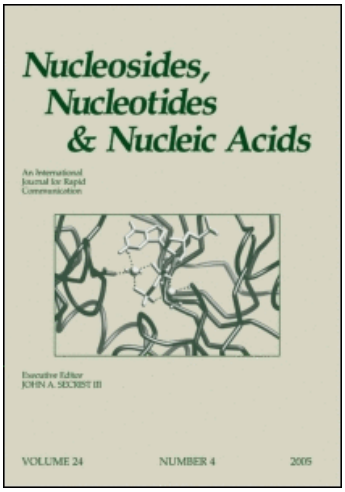

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Carbocyclic Nucleotides as Potential Substrates for Thymidylate Kinase

O. R. Ludek[a]; C. Meier[a]
[a] Institute of Organic Chemistry, University of Hamburg, Hamburg, Germany

To cite this Article Ludek, O. R. and Meier, C.(2005) 'Synthesis of Carbocyclic Nucleotides as Potential Substrates for Thymidylate Kinase', Nucleosides, Nucleotides and Nucleic Acids, 24: 5, 683 — 686
To link to this Article: DOI: 10.1081/NCN-200060251
URL: http://dx.doi.org/10.1081/NCN-200060251

Nucleosides, Nucleotides, and Nucleic Acids, 24 (5–7):683–686, (2005)

ISSN: 1525-7770 print/ 1532-2335 online
DOI: 10.1081/NCN-200060251

Taylor & Francis
Taylor & Francis Group

SYNTHESIS OF CARBOCYCLIC NUCLEOTIDES AS POTENTIAL SUBSTRATES FOR THYMIDYLATE KINASE

O. R. Ludek and C. Meier ▫ *Institute of Organic Chemistry, University of Hamburg, Hamburg, Germany*

▫ *Enantiomerically pure carbocyclic 2′-deoxy-3′-azidothymidine monophosphate (AZTMP) and carba-2′deoxy-3′-thiocyanatothymidine monophosphate were synthesized to study their behavior toward their phosphorylation by thymidylate kinase. The nucleotides were synthesized starting from the parent nucleosides by an alkaline hydrolysis of the corresponding cycloSal-phosphate triesters.*

Keywords Carbocyclic Nucleosides, Mitsunobu Reaction, *cyclo*Sal-Pronucleotides, Nucleotides

INTRODUCTION

Nucleoside analogues used in antiviral or antitumor therapies need to be activated intracellularly by an enzymatic phosphorylation cascade in order to be biologically active. Unfortunately, some of the well-established active nucleosides are only poor substrates for these activating enzymes. For example, d4T is a bad substrate in the first phosphorylating step, catalyzed by thymidine kinase, whereas AZT is only phosphorylated inefficiently from the monophosphate to the diphosphate by thymidylate kinase.[1] In the case of d4T, this kinetic bottleneck can be successfully circumvented by using an established pronucleotide approach, e.g., the *cyclo*Sal-concept (thymidine kinase bypass).[2] So far, this strategy could not be applied to the intracellular activation of AZT.

The reason for the slow conversion from AZTMP to AZTDP is a structural change of the enzyme thymidylate kinase while binding the unnatural substrate AZT-monophosphate. The bulky 3′-azido-group of AZTMP causes a shift in the p-loop region, leading to an almost complete breakdown of the catalytic mechanism, responsible for the phosphoryl group transfer.[3]

Address correspondence to C. Meier, Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, Hamburg D-20146, Germany.

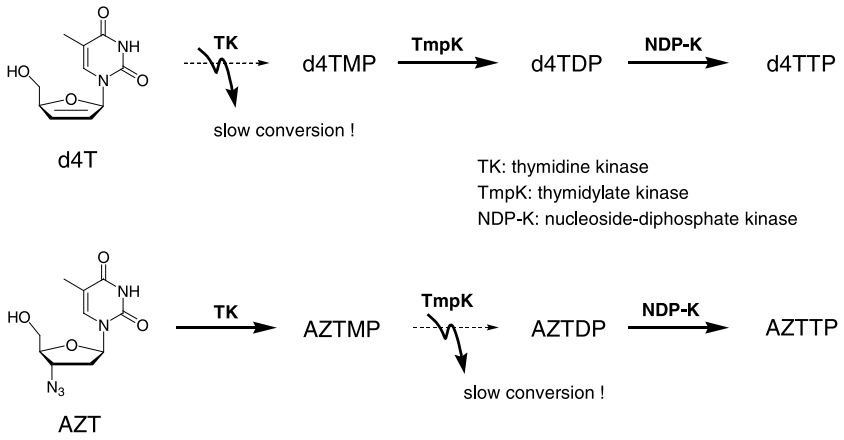

FIGURE 1 Phosphorylation cascade of antivirally active nucleosides.

RESULTS

In our group, we are interested in investigating the structural requirements of nucleotides phosphorylation efficiently by thymidylate kinase. In a first attempt we replaced the azido group in AZTMP by isosteric substituents, e.g., the thiocyanato-, the allyl-, and propargyl group, and we increased the flexibility of the nucleotide by using acyclic analogues of thymidine.[4] Surprisingly, none of the prepared nucleotides were substrates for TmpK. Apparently, the acyclic nucleotides were too flexible to bind to the active site of the kinase.

For the synthesis of carbocyclic 3′-azido-2′-deoxythymidine monophosphate (*carba*-AZTMP) and *carba*-3′-thiocyanato-2′deoxythymidine monophosphate, we started from enantiomerically pure carbocyclic thymidine **1**, which was prepared by a condensation reaction of the corresponding protected cyclopentanol with the protected nucleobase under Mitsunobu conditions.[5] As reported before, besides

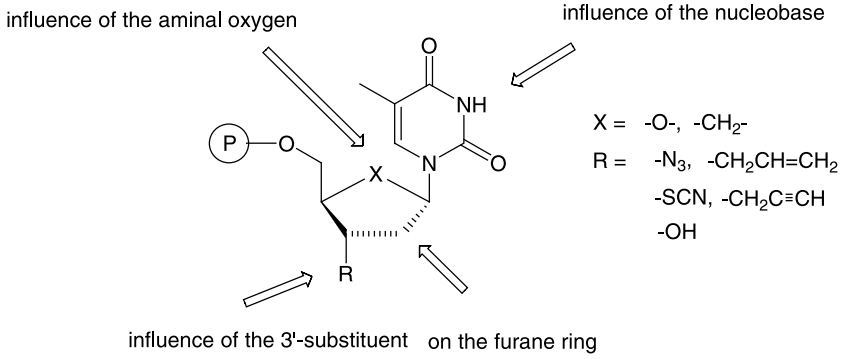

FIGURE 2 Variable parameters for the SAR of nucleotides towards thymidylate kinase.

the desired *N*-1-alkylated product, the undesired *O*-2-alkylated product is formed. The ratio of these two products can be fine-tuned by variation of the solvents and the substituents on the benzoyl group in the *N*-3-position of the nucleobase. We found that nonpolar solvents (1,4-dioxane, diethylether) and electron-donating groups in the benzoyl residue favour the *O*-2-alkylation, while polar solvents (acetonitrile) and electron-withdrawing substituents lead predominantly to the desired *N*-1-product. We also found a strong dependence of the product ratio on the electron density on the carbon bearing the hydroxy group. The nucleobase can react as an ambident nucleophile with a hard (O) and a soft (N) alkylation site. The less electron density (highfield shift in ^{13}C NMR), the more *O*-2-alkylated product can be isolated.

After tritylation of the 5′-hydroxy group in *carba*-dT **1** with triphenylmethylchloride in pyridine, the protected nucleoside **2** was converted into the 2,3′-anhydro derivative **3** under Mitsunobu conditions (90% yield). Nucleophilic opening of the anhydro structure with sodium azide in DMF gave the protected target structure (82% yield). After detritylation with 2% benzensulfonic acid (BSA) in dichloromethane/methanol (7:3) in 79% yield, the desired carbocyclic AZT **5** was obtained. For the synthesis of the thiocyanato derivative **6**, a second portion of the protected 2,3′-anhydro structure **3** was hydrolyzed quantitativly to the corresponding *xylo*-nucleoside **4** with sodium hydroxide in ethanol. To our surprise, after activation of the 3′-hydroxy group by triflic anhydride in dichloromethane and subsequent nucleophilic displacement by sodium thiocyante in DMF, the protected target structure could only be obtained in 12% yield.[6] Detritylation led to the desired 3′-thiocyanato derivative **6** in 79% yield.

Finally, the nucleosides were transferred into their monophosphate esters (nucleotides) by an alkaline hydrolysis of the corresponding *cyclo*Sal-phosphate triesters. The *cyclo*Sal pronucleotide system was originally designed for a selective intracellular delivery of therapeutically active nucleoside monophosphates by a purely pH-driven mechanism, but it can also be used for the synthesis of nucleotides in the laboratory. The synthesis of the *cyclo*Sal-phosphate triesters was performed

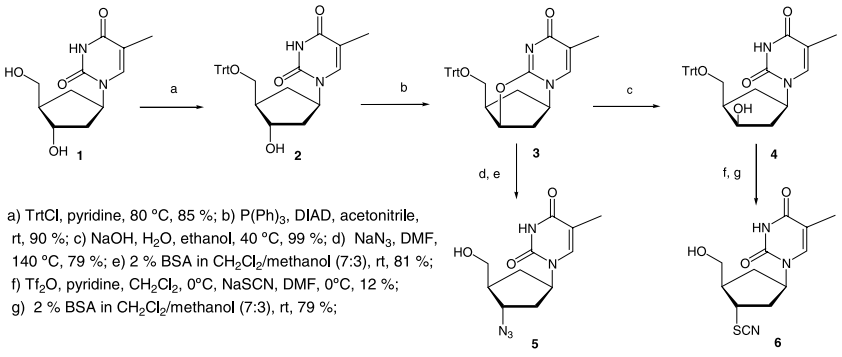

FIGURE 3 Synthesis of 3′-modified carbocyclic nucleosides.

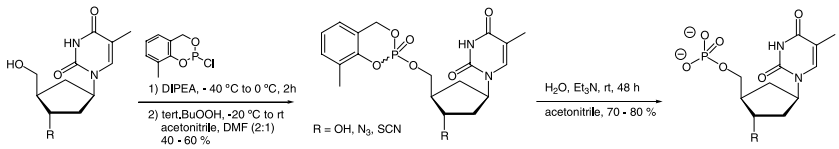

FIGURE 4 Synthesis of carbocyclic nucleoside 5′-monophosphates.

using acetonitrile/DMF and the chlorophosphite/*t*-butylhydroperoxide method at −20°C, as published before.[7] These phosphate triesters were then dissolved in actetonitrile/water and triethylamine was added. The reaction mixture was stirred until a complete decomposition of the educt was observed.

REFERENCES

1. Balzarini, J.; Naesens, L.; Aquaro, S.; Knispel, T.; Perno, C.-F.; De Clercq, E.; Meier, C. Intracellular metabolism of *cyclo*Saligenyl 3′-azido-2′,3′-dideoxy-thymidine monophosphate, a prodrug of 3′-azido-2′,3′-dideoxythymidine (zidovudine). Mol. Pharmacol. **1999**, *56*, 1354–1361.
2. Meier, C. *cyclo*Sal-pronucleotides—design of chemical trojan horses. Mini Rev. Med. Chem. **2002**, *2*, 219–234.
3. Lavie, A.; Schlichting, I.; Vetter, I.R.; Konrad, M.; Reinstein, J.; Goody, R.S. The bottleneck in AZT activation. Nat. Med. **1997**, *3*, 922–924.
4. Müller, H.C.; Meier, C.; Balzarini, J.; Reinstein, J. Novel nucleotide analogues as potential substrates for TmpK—a key enzyme in the metabolism of AZT. Nucleosides Nucleotides Nucleic Acids **2003**, *5–8*, 821–823.
5. Ludek, O.R.; Meier, C. New convergent synthesis of carbocyclic nucleoside analogues. Synthesis **2003**, *13*, 2101–2109.
6. Schreiber, S.L.; Ikemoto, N. Synthesis of chemically reactive analogues of AZT and their biological evaluation against HIV. Tetrahedron Lett. **1988**, *28*, 3211–3214.
7. Meier, C. 4H-[1.3.2]-benzodioxaphosphorin-2-nucleosyl-2-oxide—a new concept for lipophilic, potential prodrugs of biologically active nucleoside monophosphates. Angew. Chem., Int. Ed. Engl. **1996**, *35*, 70–72.